Gene, 135 (1993) 9–13

GENE 07414

Co-chairman's remarks: before the double helix*

(Proteins; DNA; Avery; Watson; Crick)

Max F. Perutz

MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK

Received by G. Bernardi: 19 May 1993; Accepted: 3 June 1993; Received at publishers: 16 July 1993

'Of course, now that we know the answer, it seems so completely obvious that no-one nowadays remembers just how puzzling the problem seemed then'
(Crick, 1988)

SUMMARY

In the nineteen-thirties and -forties, genes were universally believed to be made of protein. Biochemists met Avery, MacLeod and McCarty's discovery, that the transforming factor of pneumococci consists of DNA, with disbelief, and the notion that this was true of genes took a long time to be generally accepted even after Watson and Crick's discovery of its double helical structure. Until Watson's arrival, Kendrew, Crick and I were interested mainly in solving the structure of proteins, but Watson made us think about the structure of genes which determine protein structure.

In 1936, I left my hometown Vienna, Austria, for Cambridge, England, to seek the Great Sage. I asked him: 'How can I solve the secret of life?' He replied: 'The secret of life lies in the structure of proteins, and X-ray crystallography is the only way to solve it'. The Sage was John Desmond Bernal, a flamboyant Irishman who headed the Crystallographic Department of the Cavendish Laboratory and who had been the first to discover that protein crystals give detailed X-ray diffraction patterns extending to spacings of the order of interatomic distances (Bernal and Crowfoot, 1934). We really called him Sage, because he knew everything. During a discourse at the Royal Institution in London Bernal said: 'The structure of proteins is the major unsolved problem at the boundary of chemistry and biology today. It is difficult to exaggerate the importance of this study to many branches of science. The protein is the key unit in biochemistry and physiology. ... All protein molecules that we know now have been made by other protein molecules, and these in turn by others' (Bernal, 1939). When Bernal advanced this argument in a later BBC discussion, the physicist W.H. Bragg asked him where the first protein had come from. Instead of replying 'I don't know', Bernal skilfully sidestepped Bragg's awkward question. Inspired by Bernal's enthusiasm, I became a crystallographer and began to work on the structure of haemoglobin, because it was the protein that was most abundant and easiest to crystallise.

What had attracted me to Cambridge as an undergraduate in Vienna was Gowland Hopkins' work on vitamins and enzymes. He was the founder of Cambridge biochemistry. In the nineteen-thirties he still had had to battle against vitalism to get acceptance for his then heretical view that 'The living cell, at one definite level of its organisation, admitting that higher levels may be superimposed, is to be pictured as the seat of diverse but organised chemical reactions, in which substances identifiable by chemical methods undergo changes which can be followed by chemical methods. The molecules of these substances are activated and their reactions directed in space and time by the catalytic agencies which are commonly known as intracellular enzymes. The influence of

Correspondence to: Dr. M.F. Perutz, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, UK. Tel. (44-223) 248011; Fax (44-223) 213556.

*Presented at the COGENE Symposium, 'From the Double Helix to the Human Genome: 40 Years of Molecular Genetics', UNESCO, Paris, 21–23 April 1993.

these differs in no essentials from that of catalysts in non-living systems save that it is displayed in relations which are exceptionally specific' (Hopkins, 1932). Even today some philosophers would reject Hopkins' views as *reductionist*.

Hopkins was the professor of biochemistry. The reader was J.B.S. Haldane, a devoted communist in the guise of an English squire, and one of the most imaginative scientists of his generation. He pointed out that many enzymatic reactions as well as blood groups are genetically controlled.

'Two possibilities are now open. The gene is a catalyst making a particular antigen, or the antigen is simply the gene or part of it let loose from its connection with the chromosome. The gene has two properties. It intervenes in metabolism, sometimes at least by making a definite substance. And it reproduces itself. The gene, considered as a molecule, must be spread out in a layer one building block deep. Otherwise it could not be copied. The most likely method of copying is by a process analogous to crystallization, a second similar layer of building block being laid down on the first. *But we could conceive of a process analogous to the copying of a gramophone record by the intermediation of a "negative" perhaps related to the original as an antibody to an antigen*' (Haldane, 1937) (author's italics).

Haldane dismissed the idea that genes might be made of nucleic acids and asserted that the most likely substances are the histones, which are proteins.

Three years later Pauling and Delbrück published a seminal paper. It was an attack on the German theoretician Pascual Jordan, who had advanced the idea that there exists a quantum-mechanical stabilizing interaction, operating preferentially between identical and near-identical molecules, which governs biological processes such as the reproduction of genes (Jordan, 1938). Pauling and Delbrück pointed out that interactions between molecules were now rather well understood to give stability to two molecules of *complementary* structure in juxtaposition, rather than two molecules with necessarily *identical* structures (Pauling and Delbrück, 1940).

In 1944 the almost universally held view that genes consist of proteins was overturned by Avery, MacLeod and McCarty's discovery that the transforming principle of pneumococci is made of DNA. Oswald Avery, who took the first step as a young man and persevered until his retirement, was an even more reluctant revolutionary in biology than Max Planck had been in physics earlier in the century. He was born in Halifax, Nova Scotia in 1877, the son of a Baptist Minister who had emigrated there from England and later became pastor of a Baptist church in a poor district of New York.

Motivated by his family's Christian missionary background Oswald Avery became a doctor, but soon abandoned practice for research. This remained humdrum until, aged 36, he was appointed bacteriologist to the hospital of the Rockefeller Institute for Medical Research in New York. At that time lumbar pneumonia killed 50 000 people a year in the United States. Avery's mother had died of it. Avery wondered why it killed some people, while others recovered.

The first pointers came in 1916. Avery's friend A.R. Dochez discovered in the filtrate of a pneumococcus culture a *specific soluble substance* that flocculated in an antiserum against the type of pneumococcus growing in the culture. Dochez and Avery then found that same substance in the blood and urine of their patients and concluded, wrongly as it turned out, that it was a protein. In succeeding years Avery convinced himself that it was the same substance which formed the bacterial capsule originally described by Neufeld in Robert Koch's laboratory in Berlin.

Avery sensed that this *specific soluble substance* played a vital part in the disease and wanted to find out more about it. When the organic chemist Michael Heidelberger joined the Institute, he used to agitate a tube of it in front of him saying: 'Michael, the whole secret of bacterial specificity is in this tube. When can you work on it?' When Heidelberger finally did take it up, he found it to consist, not of protein, but of polysaccharide (Avery and Heidelberger, 1923).

The previous year Fred Griffith at the laboratory of the Ministry of Health in London had discovered the nature of the difference between virulent and non-virulent pneumococci that Avery had sought for so long. He found that the virulent cocci were encapsulated and the non-virulent ones were not. Griffith called the encapsulated ones smooth and the others rough. So the *specific soluble substance* of the capsule consisted of polysaccharide and was associated with virulence.

Heidelberger and Avery tried to make it produce an antiserum. They failed, but Avery did make an effective antiserum by immunizing horses with virulent pneumococci, and this saved many lives.

The next great advance came in 1928 with a startling discovery by Griffith. He injected into mice a mixture of live rough pneumococci of type I and heat-killed smooth pneumococci of type II. This mixture killed the mice, even though the rough pneumococci were non-virulent. The reason became apparent when Griffith isolated from their blood live smooth pneumococci of type II. Their capsular shell contained polysaccharide of type II, showing that the live rough pneumococci of type I had been transformed into live ones of type II! (Griffith, 1928). Griffith interpretated the transformation as a Lamarckian kind of adaptation. 'When the R form of either type is fur-

nished under suitable experimental conditions with a mass of the S form of the other type, it appears to utilize that antigen as a pabulum (food) from which to build up a similar antigen and thus it develops into an S strain of that type' (Griffith, 1928). He thought that the recipient rough cocci had retained the power to synthesize the capsular polysaccharides of several serological types and needed only the specific stimulus of the killed encapsulated cells to adapt themselves and make the polysaccharide capsule again. Apparently he never thought of mutations, perhaps because bacteria were then believed not to exhibit genetics.

According to his colleague René Dubos, Avery refused to believe Griffith's results until another member of the Institute, Henry Dawson, repeated them while Avery was away sick (Dubos, 1976). Dawson and Sia did away with the mice and transformed rough pneumococci into smooth ones in cultures in beef broth. After this Avery was convinced and used to ask persistently, day after day, year after year: 'What is the substance responsible for the transformation?', but he was handicapped by being a bacteriologist rather than a biochemist.

After Dawson had left the Institute in 1930, Avery encouraged his successor J.L. Alloway to pursue the problem. He dissolved the virulent cocci in deoxycholate, filtered off the cellular debris and found the solution to be active. The activity came down as a thick, syrupy precipitate on addition of alcohol and could be redissolved in water without loss of activity but, unbelievably, it took another 13 years to find out what this precipitate was made of. Not even Colin MacLeod's pregnant observation, made in 1936, that the transforming activity was destroyed by ultraviolet light gave the clue.

The slow progress was due partly to the very inconsistent yields of transforming activity extracted from the pneumococci, due to its variable destruction by bacterial enzymes. It was not until 1941 that Avery surmounted this trouble by heating the transformed cocci to 65°C for 30 min *before* lysing them with deoxycholate. This inactivated the bacterial enzymes that had destroyed the activity, and left the transforming principle intact.

The final attack started in September 1941 with the arrival of Maclyn McCarty, a young medical doctor with a good biochemical training. It then seemed most likely that the transforming principle was a protein, whence enzymes that digest proteins should have destroyed it. Nowadays such enzymes can be bought off the shelf, but in those days McCarty was lucky to be given some trypsin and chymotrypsin by Northrop at the Rockefeller Institute in Princeton who had been the first to isolate and crystallise them in pure form.

Northrop's enzymes left the transforming activity intact. Could it reside in RNA? Northrop's colleague Moses Kunitz had just crystallised ribonuclease and gave Avery some of his crystals. Ribonuclease also left the transforming activity intact. The same held for enzymes that hydrolyse polysaccharides. Avery and McCarty's therefore set out to rid an extract of the virulent bacteria of protein, RNA and polysaccharides without destroying the transforming activity, and then isolating that activity in pure form.

After a lot of hard work McCarty obtained a goocy precipitate of white fibres which took up a stain characteristic of DNA and showed properties similar to the DNA that another member of the Rockefeller Institute, Alfred Mirsky, had isolated from calf thymus. Now came the crucial test. Would the transforming activity be destroyed by deoxyribonuclease? No-one could provide this. McCarty had to isolate it laboriously from dog intestinal mucosa, or swine kidneys, or rabbit blood. He made sure that it degraded neither protein, nor RNA, nor polysaccharide, but it did destroy the transforming activity (McCarty, 1985). Avery and McCarty now piled proof upon proof to convince themselves of their finding, yet it was so revolutionary that it took Avery a long time before he finally summoned the courage to publish it. The paper is absolutely rigorous and leaves no shadow of doubt that the transforming factor consists of DNA and nothing but DNA.

Did Avery comprehend the full significance of his discovery? Rollin Hotchkiss who worked with him, has testified that Avery 'was well aware of the implications of DNA transforming agents for genetics and infections'. McFarlane Burnett, who visited Avery in 1943, wrote home to his wife that Avery 'had just made an extremely exciting discovery which, put rather crudely, is nothing less than the isolation of a pure gene in the form of DNA' (Olby, 1974). It was a revolutionary finding, but Avery was no revolutionary. He was a small, delicate monkish bachelor who lived only for his science, and for his life's aim to find the cause and cure of virulent pneumonia. He wore pince-nez, was fastidious with his words, ever cautious in his public utterances, never went on lecture tours, wrote no books and never travelled. He never co-authored any paper on research to which he had not actively contributed, did not patent his discovery and never became rich.

In 1944 genes were still almost universally believed to be made of protein. Aaron Levene, a chemist at the Rockefeller Institute, had proposed that all DNAs are made up of regular sequences of the same four nucleotides, whence they could not carry information. Alfred Mirsky, who had spent years working on DNA without realizing its significance, was sure that the transforming activity was carried by protein impurities in Avery's DNA. As late as 1947, Mirsky said at Cold Spring

Harbor: 'In the present state of knowledge, it would be going beyond the experimental facts to assert that the specific agent in transforming bacterial types is DNA' (McCarty, 1985). Seeing that as little as 3 ng of DNA had been sufficient to transform the cocci in half an assembly of infected test tubes, the idea that transformation could have been effected by a protein impurity seems far fetched. Perhaps because of Mirsky's continued smear campaign, Avery, MacLeod and McCarty never received the Nobel Prize for one of the century's greatest discoveries. Yet to its credit, the Royal Society recognised the discovery by making Avery a Foreign Member and awarding him their highest honour, the Copley Medal. Sir Henry Dale, the President, said in his citation: 'Here surely is a change to which, if we were dealing with higher organisms, we should accord the status of a genetic variation; and the substance inducing it – the gene in solution, one is tempted to call it – appears to be a nucleic acid of the deoxyribose type' (Dale, 1946).

Robert Olby concludes his authoritative account of the history of the transforming factor with the words: 'With the passage of time the work of Avery, MacLeod and McCarty looks, if anything, more significant than in 1953; perhaps it was the most important discovery in the path to the double helix' (Olby, 1974), but most people remained sceptical for many years afterwards. Some were convinced when Hotchkiss (1951) transferred penicillin resistance to a non-resistant strain of pneumococci with DNA from a resistant strain. Others dropped their doubts when Alfred Hershey and Martha Chase (1952) demonstrated that on phage infection only the phage DNA and not the phage protein enters *E. coli*. References to the transforming principle in successive editions of J.N. Davidson's textbook on nucleic acids illustrate the scepticism that persisted for a long time even after that.

'If the active DNA is in fact protein-free, we have here an example of a specific biological property, the ability to induce the synthesis of a characteristic immunological polysaccharide, which is peculiar to one form of DNA and no other. Not only is this the first good example of a biological activity attributable to a nucleic acid per se; it indicates that there may be important differences between one specimen of DNA and another which may not be detectable by chemical means' (Davidson, 1950; 1953).

'It seems reasonable therefore to interpret bacterial transformation as indicating that DNA is the active material of the gene; ... It has proved a matter of some difficulty to find evidence in confirmation of that hypothesis' (Davidson, 1960; 1963).

Davidson did not specify the nature of that difficulty.

I was still submerged in haemoglobin when one day in September 1951 a strange young head with a crew-cut and bulging eyes popped through my door and asked, without saying as much as hello, 'Can I come and [illegible] here?' He was Jim Watson, who wanted to join the small team of enthusiasts for molecular biology at the Cavendish Laboratory in Cambridge which I led.

My colleagues were John Kendrew, a chemist like myself, and Francis Crick and Hugh Huxley, both physicists. We shared the belief that the nature of life could be understood only by getting to know the atomic structure of living matter, and that physics and chemistry would open the way, if only we could find it.

In his best-selling book 'The Double Helix' Watson mirrors himself as a brash western cowboy entering our genteel circle, but this is a caricature. Watson's arrival had an electrifying effect on us because he made us look at our problems from the genetic point of view. He asked not just: what is the atomic structure of living matter? but, foremost, what is the structure of the gene that determines it? Watson found an echo in Crick who had begun to think along similar lines. Crick was 34, a more than mature graduate student due to years lost by the war; Watson was 22, a whizz-kid from Chicago who had entered university aged 15, and got his Ph.D. in genetics at 20.

They shared the sublime arrogance of men who had rarely met their intellectual equals. Crick was tall, fair, dandyishly dressed and talked volubly, each phrase in his King's English strongly accented and punctuated by eruptions of jovial laughter that reverberated through the laboratory. To emphasise the contrast, Watson went around like a tramp, making a show of not cleaning his one pair of shoes for an entire term (an eccentricity in those days), and dropped his sporadic nasal utterances in a low monotone that faded before the end of each sentence, and was followed by a snort.

To say that they did not suffer fools gladly would be an understatement: Crick's comments would hit out like daggers at *non sequiturs* and Watson demonstratively unfolded his newspaper at seminars that bored him. Watson had put Crick's mind to the structure of DNA, yet their relationship was something of teacher and pupil because there was little that Watson could teach Crick, but much that Crick could teach Watson. Crick had a profound understanding of that hardest of the sciences, physics, without which the structure of DNA would never have been solved. This crucial fact is obscured in Watson's 'Double Helix'. Yet Watson had an intuitive knowledge of the features that DNA ought to have if it were to make genetic sense.

At some stage there was much argument as to whether genes consist of two or three chains of DNA wound around each other. Watson lodged with a lady retired from the stage who kept a boarding house for young girls. One day she noticed him pacing restlessly and mut-

tering to himself: 'There must be two ... there must be two ...' She guessed that this referred to matters of the heart. But we knew better. He reasoned on genetic grounds that genes must be made of two chains of DNA, and he was right.

Like Leonardo, Crick and Watson often achieved most when they seemed to be working least. They did an immense amount of hard work, studying hidden away, often at night, but when you saw them they were more likely engaged in argument and apparently idle. This was their way of attacking a problem that could be solved only by a tremendous leap of the imagination, supported by profound knowledge. Imagination comes first in both artistic and scientific creations. But in science Nature always looks over your shoulder. To paraphrase Winston Churchill: 'In science you don't need to be polite, you only have to be right'.

REFERENCES

Avery, O.T. and Heidelberger, M.: Immunological relationships of cell constituents of pneumococcus. J. Exp. Med. 38 (1923) 81–85.

Avery, O.T., MacLeod, C.M. and McCarty, M.: Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J. Exp. Med. 79 (1944) 137–158.

Bernal, J.D. and Crowfoot, D.: X-ray photographs of crystalline pepsin. Nature 133 (1934) 794–795.

Crick, F.: What Mad Pursuit. Basic Books Inc., New York, 1988.

Dale, H.: Presidential address. Proc. Roy. Soc. A 185 (1946) 127–243.

Davidson, J.N.: The Biochemistry of the Nucleic Acids. Methuen, London, 1950 and 1953.

Davidson, J.N.: The Biochemistry of the Nucleic Acids. Methuen, London 1960 and 1963.

Dubos, R.: The Professor, The Institute and DNA. Rockefeller University Press, New York, 1976.

Griffith, F.: The significance of pneumococcal types. J. Hyg. (Cambridge, UK) 27 (1928) 113–159.

Haldane, J.B.S.: The biochemistry of the individual. In: Needham, J. and Green, D.E. (Eds.), Perspectives in Biochemistry. Cambridge University Press, Cambridge, 1937, pp. 1–10.

Hershey, A.D. and Chase, M.: Independent functions of viral protein and nucleic acid in growth of bacteriophage. J. Gen. Physiol. 36 (1952) 39–52.

Hopkins, F.G.: Some aspects of biochemistry: the organising capacities of specific catalysts. Second Purser Memorial Lecture 1932. In: Needham, J. and Baldwin, E. (Eds.), Hopkins and Biochemistry. W. Heffer, Cambridge, 1949, pp. 226–242.

Hotchkiss, R.D.: Transfer of penicillin resistance in pneumococci by the deoxyribonucleate derived from resistant cultures. Cold Spring Harbor Symp. Quant. Biol. 16 (1951) 457–460.

Jordan, P.: Zur Frage einer spezifischen Anziehung zwischen den Molekülen. Phys. Z. 39 (1938) 711–714.

McCarty, M.: The Transforming Principle. W.W. Norton, New York, 1985.

Olby, R.: The Path to the Double Helix. MacMillan, London, 1974.

Pauling, L. and Delbrück, M.: The nature of the intermolecular forces operative in biological systems. Science 92 (1940) 77–79.